

2

Figure 6: Synthetic ODN cannot be mixed with DNA vaccine due to interference with expression from plasmid. The figure shows the effect of adding S-ODN to plasmid DNA expressing reporter gene or antigen. ODN 1826 (10 or 100 µg) was added to DNA constructs (10 µg) encoding hepatitis B surface antigen (HBsAg) (pCMV-S, top panel) or luciferase (pCMV-luc, bottom panel) DNA prior to intramuscular (IM) injection into mice. There was an ODN dose-dependent reduction in the induction of antibodies against HBsAg (anti-HBs, end-point dilution titers at 4 wk) by the pCMV-S DNA (top panel) and in the amount of luciferase expressed in relative light units per sec per mg protein (RLU/sec/mg protein at 3 days) from the pCMV-luc DNA (bottom panel). This suggests that the lower humoral response with DNA vaccine plus ODN was due to decreased antigen expression. Each bar represents the mean of values derived from 10 animals (top panel) or 10 muscles (bottom panel) and vertical lines represent the SEM. Numbers below the bars indicate proportion of animals responding to the DNA vaccine (top panel); all muscles injected with pCMV-luc expressed luciferase (bottom panel).

Please re-write the paragraph beginning on page 6, line 13, as follows:

Figure 7: Interference of ODN with pDNA due to backbone and sequence. The figure shows the interference of ODN with plasmid DNA depends on backbone and sequence. Luciferase activity (RLU/sec/mg protein) in mouse muscles 3 days after they were injected with 10 µg pCMV-luc DNA to which had been added no ODN (none = white bar) or 100 µg of an ODN, which had one of three backbones: phosphorothioate (S = left slanted bars: 1628, 1826, 1911, 1982, 2001 and 2017), phosphodiester (O = thick left slanted bar: 2061), or a phosphorothioate-phosphodiester chimera (SOS = right slanted bars: 1585, 1844, 1972, 1980, 1981, 2018, 2021, 2022, 2023 and 2042). Three S-ODN (1911, 1982 and 2017) and two SOS-ODN (1972 and 2042) did not contain any immunostimulatory CpG motifs. One S-ODN (1628) and three SOS-ODN (1585, 1972, 1981) had poly-G ends and one SOS-ODN (2042) had a poly-G center. The (*) indicates ODN of identical sequence but different backbone: 1826 (S-ODN), 1980 (SOS-ODN) and 2061 (O-ODN). All S-ODN (both CpG and non-CpG) resulted in decreased luciferase activity whereas SOS-ODN did not unless they had poly-G sequences.

Please re-write the paragraph beginning on page 6, line 25, as follows:

Figure 8: Temporal and spatial separation of CpG ODN and plasmid DNA. The figure shows the effect of temporal or spatial separation of plasmid DNA and S-ODN on gene

expression. Luciferase activity (RLU/sec/mg protein) in mouse muscles 3 or 14 days after they were injected with 10 μ g pCMV-luc DNA. Some animals also received 10 μ g CpG-S ODN which was mixed with the DNA vaccine or was given at the same time but at a different site, or was given 4 days prior to or 7 days after the DNA vaccine. Only when the ODN was mixed directly with the DNA vaccine did it interfere with gene expression.

Please re-write the paragraph beginning on page 7, line 6, as follows:

Figure 9: Immunization of BALB/c mice with CpG-optimized DNA vaccines. The figure shows the enhancement of *in vivo* immune effects with optimized DNA vaccines. Mice were injected with 10 μ g of pUK-S, pMAS-S, pMCG16-S or pMCG50-S plasmid DNA bilaterally (50 μ l at 0.1 mg/ml in saline) into the TA muscle. The top panel shows the anti-HBs antibody response at 6 weeks (detected as described in methods). Bars represent the group means (n=5) for ELISA end-point dilution titers (performed in triplicate), and vertical lines represent the standard errors of the mean. The numbers on the bars indicate the ratio of IgG2a:IgG1 antibodies at 4 weeks, as determined in separate assays (also in triplicate) using pooled plasma. The bottom panel shows the cytotoxic T lymphocyte activity in specifically restimulated (5 d) splenocytes taken from mice 8 wk after DNA immunization. Bars represent the group means (n=3) for % specific lysis (performed in triplicate) at an effector:target (E:T) ratio of 10:1, dots represent the individual values. Non-specific lytic activity determined with non-antigen-presenting target cells, which never exceeds 10%, has been subtracted from values with HBsAg-expressing target cells to obtain % specific lysis values.

Please re-write the paragraph beginning on page 7, line 19, as follows:

Figure 10 shows induction of a Th2-like response by a CpG-N motif and inhibition of the Th1-like response induced by a CpG-S motif. Anti-HBs antibody titers (IgG1 and IgG2a subclasses) in BALB/c mice 12 weeks after IM immunization with recombinant HBsAg, which was given alone (none) or with 10 μ g stimulatory ODN (1826), 10 μ g of neutralizing ODN (1631, CGCGCGCGCGCGCGCGCG (SEQ ID NO:22); 1984, TCCATGCCCGTTCTGCCGTT (SEQ ID NO:78); or 2010 GCGGCGGGCGGCGCGCGCCC (SEQ ID NO:75); CpG dinucleotides are underlined for clarity) or with 10 μ g stimulatory ODN + 10 μ g neutralizing ODN. To improve nuclease resistance for these *in vivo* experiments, all ODN were phosphorothioate-modified. Each bar represents the group mean (n=10 for none; n=15 for #1826 and n=5 for all other groups) for anti-HBs antibody titers as determined by end-point dilution ELISA assay. Hatched portions

of bars indicate antibodies of IgG1 subclass (Th2-like) and white portions indicate IgG2a subclass (Th1-like). The numbers above each bar indicate the IgG2a/IgG1 ratio where a ratio >1 indicates a predominantly Th1-like response and a ratio <1 indicates a predominantly Th2-like response (a value of 0 indicates a complete absence of IgG2a antibodies).

Please re-write paragraph beginning on page 8, line 5, as follows:

Figure 11 shows enhancement of *in vivo* immune effects with optimized DNA vaccines. Mice were injected with 10 µg of pUK-S (white bars), pMAS-S (right slanted bars), pMCG16-S (thin right slanted bars) or pMCG50-S (left slanted bars) plasmid DNA bilaterally (50 µl at 0.1 mg/ml in saline) into the TA muscle. Panel A: The anti-HBs antibody response at 6 weeks (detected as described in methods). Bars represent the group means (n=5) for ELISA end-point dilution titers (performed in triplicate), and vertical lines represent the standard errors of the mean. The numbers on the bars indicate the ratio of IgG2a:IgG1 antibodies at 4 weeks, as determined in separate assays (also in triplicate) using pooled plasma. Panel B: Cytotoxic T lymphocyte activity in specifically restimulated (5 d) splenocytes taken from mice 8 wk after DNA immunization. Bars represent the group means (n=3) for % specific lysis (performed in triplicate) at an effector: target (E:T) ratio of 10:1, dots represent the individual values. Non-specific lytic activity determined with non-antigen-presenting target cells, which never exceeds 10%, has been subtracted from values with HBsAg-expressing target cells to obtain % specific lysis values.

Please re-write the paragraph beginning on page 35, line 8, as follows:

(i) Insertion of the CMV (human cytomegalovirus) major intermediate early promoter/enhancer region

The CMV promoter (from pcDNA3 position 209 to 863) was amplified by PCR using 30 ng pcDNA3 as a template. The forward PCR primer 5'CGT GGA TAT CCG ATG TAC GGG CCA GAT AT 3'(SEQ ID NO:4) introduced an EcoRV site, and the reverse PCR primer 5' AGT CGC GGC CGC AAT TTC GAT AAG CCA GTA AG 3'(SEQ ID NO:5) introduced a *Not*I site. After digestion with EcoRV and *Not*I, a 0.7 kb PCR fragment containing the CMV promoter was purified and inserted into the pUK21 polylinker between *Xba*I and *Not*I sites. The *Xba*I sticky end of pUK21 was filled in with the large fragment of T4 DNA polymerase after digestion to create a blunt end. The inserted CMV promoter was confirmed by sequencing. The resulting plasmid was pUK21-A1 (Figures 1A and 1B).

Please re-write the paragraph beginning on page 35, line 19, as follows:

(ii) Insertion of the BGH polyA (bovine growth hormone polyadenylation signal)

BGH polyA (from pcDNA3 position 1018 to 1249) was amplified by PCR using pcDNA3 as template. The forward PCR primer 5' ATT CTC GAG TCT AGA CTA GAG CTC GCT GAT CAG CC 3' (SEQ ID NO:6) introduced *Xho*I and *Xba*I sites, and the reverse PCR primer 5' ATT AGG CCT TCC CCA GCA TGC CTG CTA TT 3' (SEQ ID NO:7) introduced a *Stu*I site. After digestion with *Xho*I and *Stu*I, the 0.2 kb PCR fragment containing the BGH polyA was purified, and ligated with the 3.7 kb *Xho*I-*Stu*I fragment of pUK21-A1. The inserted BGH polyA was confirmed by sequencing. The resulting plasmid was pUK21-A2 (Figures 2A and 2B).

Please re-write the paragraph beginning on page 36, line 24, as follows:

(i) Insertion of the fl origin of replication region

The fl origin and two unique restriction enzyme sites (*Dra*I and *Apal*) were introduced into pUK21-A2 for later vector construction. fl origin (from pcDNA3 position 1313 to 1729) was amplified by PCR using pcDNA3 as template. The forward PCR primer 5' TAT AGG CCC TAT TTT AAA CGC GCC CTG TAG CGG CGC A 3' (SEQ ID NO:8) introduced *Eco*O109I and *Dra*I sites, and the reverse PCR primer 5' CTA TGG CGC CTT GGG CCC AAT TTT TGT TAA ATC AGC TC 3' (SEQ ID NO:9) introduced *Nar*I and *Apal* site. After digestion with *Nar*I and *Eco*O109I, the 0.4 kb PCR fragment containing the fl origin was purified and ligated with the 3.3 kb *Nar*I-*Eco*O109I fragment of pUK21-A2, resulting in pUK21-A (Figures 3A and 3B).

Please re-write the paragraph beginning on page 38, line 22, as follows:

(iii) Replacement of the fl origin with unique restriction enzyme sites

Oligonucleotides 5' AAA TTC GAA AGT ACT GGA CCT GTT AAC A 3' (SEQ ID NO:10) and its complementary strand 5' CGT GTT AAC AGG TCC AGT ACT TTC GAA TTT 3' (SEQ ID NO:11) were synthesized, and 5'-phosphorylated. Annealing of these two phosphorylated oligos resulted in 28 base pair double-stranded DNA containing three unique restriction enzyme sites (*Scal*, *Aval*II, *Hpa*I), one sticky end and one blunt end. Replacing the 0.4 kb *Nar*I-*Dra*I fragment of pUK21-B with this double-stranded DNA fragment resulted in the universal vector pMAS for DNA vaccine development (Figures 4A and 4B and 5).

Please re-write the paragraph beginning on page 44, line 11, as follows:

6

In contrast to the success with protein antigens, attempts to augment immune responses induced by a HBsAg-expressing DNA vaccine by the addition of CpG-S ODN 1826 failed. Surprisingly, the immune responses decreased with the addition of CpG-S ODN in a dose-dependent manner (Figure 6, top panel). Addition of ODN #1826 to a luciferase reporter gene construct (pCMV-luc, Davis *et al.*, 1993b) resulted in a dose-dependent decrease in luciferase expression (Figure 6, bottom panel). This indicates that the negative effects of the CpG-S ODN on the DNA vaccine were due to reduced gene expression rather than an effect on the immune response against the gene product.

Please re-write the paragraph beginning on page 48, line 15, as follows:

Next, different numbers of CpG-S motifs were inserted into the vector by allowing self-ligation of a 20bp DNA fragment with the sequence 5'
GACTCCATGACCGTCCATGACCGTCCATGACCGTCCATGACCGTCCCTGACGTTG 3' (SEQ ID NO:12)
with a complementary strand and inserting different numbers of copies into the *Ava*II site of pMAS. Recombinant clones were screened and the two vectors were chosen for further testing with 16 and 50 CpG-S motifs, and named pMCG16 and pMCG50 respectively.

Please re-write the paragraph beginning on page 51, line 16, as follows:

When tested for their ability to induce cytokine (IL-6 and IL-12) secretion from cultured spleen cells, we found that the pMAS-S, pMCG16-S and pMCG50-S vectors had significantly enhanced immune stimulatory activity compared to pUK-S. When used as a DNA vaccine, the anti-HBs response at 4 and 6 weeks was substantially stronger with DNA vaccines from which CpG-N motifs had been deleted, and even more so when 16 CpG-S motifs had been inserted. The vector with 50 CpG-S motifs, however, was less effective at inducing antibody production than that with 16 motifs. (Figure 11, panel A). Removal of CpG-N motifs and addition of CpG-S motifs resulted in a more than three-fold increase in the proportion of IgG2a relative to IgG1 anti-HBs antibodies, indicating an enhanced Th-1 response. This accentuated Th1 response also was demonstrated by the striking progressive increases in CTL responses induced by vectors from which CpG-N motifs were deleted and/or CpG-S motifs added (Figure 11, panel B).

Please re-write the paragraph beginning on page 53, line 20, as follows:

Based on our *in vitro* experiments we hypothesized that the presence of CpG-N motifs in DNA vaccines interferes with the induction of the desired immune response. Indeed, the

7

present study demonstrates that elimination of CpG-N motifs from a DNA vaccine leads to improved induction of antibodies. By removing 52 of the CpG-N motifs from a DNA vaccine (45 were deleted and 7 turned into CpG-S motifs) the serologic response was more than doubled; by then adding an additional 16 CpG-S motifs, the response was enhanced nearly 10 fold (Figure 11, panel A). Likewise, CTL responses were improved by removing CpG-N motifs and even more so by adding 16 or 50 CpG-S motifs (Figure 11, panel B). These increased responses are especially notable in view of the fact that the total number of CpG dinucleotides in the mutated vaccines is considerably below the original number.

Please re-write the paragraph beginning on page 54, line 2, as follows:

The finding that the vector with 50 CpG-S motifs was inferior to that with 16 motifs for induction of humoral immunity was unexpected, and may be secondary to CpG-induced production of type I-interferons, and subsequent reduction in the amount of antigen expressed. The decreased antibody response induced by pMCG50-S seems unlikely to be explained by vector instability since this vector gave the best CTL responses (Figure 11, panel B). Although the pMCG50-S vector was slightly larger than pMCG16-S, the 10 μ g dose still contained 93% as many plasmid copies as it did pMCG16-S, so lower copy number is unlikely to account for the reduced antibody levels. The current generation of DNA vaccines are quite effective in mice, but much less effective in primates (Davis, H.L., *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:7213-7218 (1996); Letvin, N.L., *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:9378-9383 (1997); Fuller, D.H., *et al.*, *J Med. Primatol.*, 25:236-241 (1996); Lu, S., *et al.*, *J Virol.*, 70:3978-3991 (1996); Liu, M.A., *et al.*, *Vaccine*, 15:909-919 (1997); Prince, A.M., *et al.*, *Vaccine*, 15:9196-919 (1997); Gramzinski, R.A., *et al.*, *Molec. Med.*, 4:109-119 (1998)). Our present results indicate that attaining the full clinical potential of DNA vaccines will require using engineered vectors in which CpG-N motifs have been deleted, and CpG-S motifs added.

Please re-write Table 1, beginning on page 56, line 22, as follows:

Table 1.

Primers used for site-directed mutagenesis.

Mutated nucleotides are underlined. Restriction enzyme sites for cloning, are indicated in bold.

Forward primers:

| | |
|-------------|--|
| Mu-0F | 5' GTCTCTAGACAGCCACTGGTAACAGGATT 3' (845) (SEQ ID NO:23) |
| Mu-1F | (1144) 5' <u>GTCGTTGT</u> TCGTCAAGTCAGCGTAATGC 3' (1172) (SEQ ID NO:24) |
| Mu-2F | (1285) 5' <u>TCGTTTCTG</u> TAATGAAGGAG 3' (1304) (SEQ ID NO:25) |
| Mu-3F | (1315) 5' <u>AAGGCAGT</u> CCATAGGATGG 3' (1334) (SEQ ID NO:26) |
| Mu-(4+5)F | (1348) 5' TCG <u>AT</u> CTGCGATT <u>CCA</u> ACTCGTCCAACATCAATAC 3' (1382) (SEQ ID NO:27) |
| Mu-6F | (1453) 5' <u>TGGTGAGA</u> ATGGCAAAAGTT 3' (1472) (SEQ ID NO:28) |
| Mu-7F | (1548) 5' CATTATT <u>CATT</u> CGTGATTGCG 3' (1568) (SEQ ID NO:29) |
| Mu-8F | (1633) 5' <u>ACGTCT</u> CAGGAACACTGCCAGCGC 3' (1656) (SEQ ID NO:30) |
| Mu-9F | (1717) 5' <u>AGGGAT</u> CGCAGTGGTGAGTA 3' (1736) (SEQ ID NO:31) |
| Mu-10F | (1759) 5' <u>TATAAA</u> ATGCTTGATGGTCGG 3' (1779) (SEQ ID NO:32) |
| Mu-(11+12)F | (1777) 5' <u>GGGAAGAGGCATAAATT</u> TCAGGCCAGTTAGTC 3' (1811) (SEQ ID NO:33) |
| Mu-13F | (1882) 5' <u>TGGCTTCCC</u> CATAAAGCGAT 3' (1901) (SEQ ID NO:34) |
| Mu-14F | (1924) 5' <u>TACATTATCGCGAGCCC</u> ATT 3' (1943) (SEQ ID NO:35) |
| Mu-15F | (1984) 5' <u>TGGCCTCGACGTTCCCGT</u> 3' (2002) (SEQ ID NO:36) |

Reverse primers:

| | |
|-----------|---|
| Mu-0R | 5' ATCGA <u>ATT</u> CAGGGCC <u>TC</u> GTGATA <u>CG</u> CCTA 3' (2160) (SEQ ID NO:37) |
| Mu-1R | (1163) 5' TGACTTGAC <u>GA</u> <u>CA</u> <u>AC</u> <u>CG</u> <u>AC</u> <u>AG</u> CTCATGACCAAA <u>ATCCC</u> 3' (1125) (SEQ ID NO:38) |
| Mu-2R | (1304) 5' CTCCTTCATTACAGAA <u>ACG</u> <u>A</u> <u>CT</u> TTTCAAAA <u>ATGGTA</u> 3' (1266) (SEQ ID NO:39) |
| Mu-3R | (1334) 5' CCATCCTATGGAA <u>CTG</u> <u>CC</u> <u>T</u> <u>GG</u> TGAG <u>TTT</u> CTCCTTC 3' (1298) (SEQ ID NO:40) |
| Mu-(4+5)R | (1367) 5' GAG <u>TTG</u> GAAT <u>CG</u> <u>CAG</u> <u>AT</u> CGATA <u>CC</u> <u>AGG</u> <u>AT</u> TTGC 3' (1334) (SEQ ID NO:41) |
| Mu-6R | (1472) 5' AACT <u>TTT</u> <u>GCC</u> <u>ATT</u> CTCAC <u>CC</u> <u>A</u> <u>G</u> ATT <u>CAG</u> <u>T</u> <u>CG</u> TC <u>ACT</u> CA 3' (1436) (SEQ ID NO:42) |
| Mu-7R | (1568) 5' CGCAAT <u>ACG</u> <u>A</u> <u>ATG</u> <u>A</u> <u>AA</u> <u>T</u> <u>GG</u> <u>TT</u> <u>GG</u> <u>TT</u> GAT <u>GC</u> <u>G</u> <u>AG</u> <u>TG</u> 3' (1530) (SEQ ID NO:43) |

Mu-8R (1652) 5' TGGCAGTGTCCCTGACGTGCATTCGATTCCCTGTT 3' (1615) (SEQ ID NO:44)

Mu-9R (1736) 5' TACTCACCACTGCGATCCCTGGAAAAACAGCATTCCAG 3' (1736) (SEQ ID NO:45)

Mu-10R (1779) 5' CCGACCATCAAGCATTTATACGTACTCCTGATGATGCA 3' (1741) (SEQ ID NO:46)

Mu-(11+12) (1796) 5' CGAATTATGCCTCTCCACCATCAAGCATTTATAC 3' (1758) (SEQ ID NO:47)

Mu-13R (1901) 5' ATCGCTTGTATGGGAAGCCAGTGCCAGAGTTGTT 3' (1882) (SEQ ID NO:48)

Mu-14R (1943) 5' AATGGGCTCGCGATAATGTAGGGCAATCAGGTGCGAC 3' (1907) (SEQ ID NO:49)

Mu-15R (2002) 5' ACGGGAAACGTCGAGGCCACGATTAAATTCCAACATGG 5' (1965) (SEQ ID NO:50)

Please re-write Table 2, beginning on page 59, line 1, as follows:

Table 2 *Nucleotide and amino acid sequences of the *AlwNI*-*EcoO109I* fragment (SEQ ID NO:80)*

| | | | | | | | |
|---------|------|---|-----------------------------|-------------------------------|-------------------------------|---------------------|---------------------|
| kan(wt) | 2180 | AAGGGCCTCG | TGATACGCCT | ATTTTTATAG | GTAAATGTCA | TGGGGGGGG | GGGGAAAGCC |
| kan(wt) | 2120 | ACGTTGTGTC | TCAAAATCTC | TGATGTTACA | TTGCACAAGA | TAAAATATA | TCATCATGAA |
| kan(wt) | 2060 | CAATAAAACT | GTCTGCTTAC | ATAAACAGTA | ATACAAGGGG | TGTTATGAGC | CATATTCAAC |
| kan(mu) | | | | | | | |
| ORF | | | | | | M S | H I Q |
| kan(wt) | 2000 | GGGAAACGTC | <u>GAGGCCGCGA</u> A | TTAAATTCCA | ACATGGATGC | TGATTTATAT | GGGTATAAAT |
| ORF | | | | | | | |
| kan(wt) | 1940 | R E T S GGGCTCGCGA | R P R TAATGTC <u>GGG</u> | L N S CAATCAGGTG | N M D A CGACAATCTA | D L Y TCGCTTGTAT | G Y K GGGAAGCCG |
| kan(mu) | | | A | | | | A |
| ORF | | | | | | | |
| kan(wt) | 1880 | W A R D ATGCGCCAGA | N V G GTTGTTCTG | Q S G AAACATGGCA | A T I Y AAGGTAGCGT | R L Y TGCCAATGAT | G K P GTTACAGATG |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1820 | D A P E AGATGGTCAG | L F L ACTAAACTGG | K H G CTGAC <u>CGGA</u> AT | K G S V TTATGCCCTCT | A N D TCCGACCATC | V T D AAGCATTAA |
| kan(mu) | | | | A | | C | |
| ORF | | | | | | | |
| kan(wt) | 1760 | E M V R TCCGTACTCC A | L N W TGATGATGCA | L T E TGGTTACTCA | F M P L CCACTGCGAT | P T I CCCCGGAAAA | K H F ACAGCATTCC |
| kan(mu) | | | | | T | | |
| ORF | | | | | | | |
| kan(wt) | 1700 | I R T P AGGTATTAGA | D D A AGAAATATCCT | W L L GATTCA <u>GGT</u> G | T T A I AAAATATTGT | P G K TGATGCGCTG | T A F GCAGTGTTC |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1640 | Q V L E TG <u>CGCCGG</u> TT A A A | E Y P GCATTGCGATT | D S G CCTGTTGTA | E N I V ATTGTCCTTT | D A L TAACAGCGAT | A V F CGCGTATTC |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1580 | L R R L GTCTCGCTCA | H S I GGCGCAATCA | P V C CGAATGAATA | N C P F ACGGTTGGT | N S D TGATGCGAGT | R V F GATTTGATG |
| kan(mu) | | | | T | | | |
| ORF | | | | | | | |
| kan(wt) | 1520 | R L A Q ACGAGCGTAA | A Q S TGGCTGGCCT | R M N GTTGAACAAG | N G L V TCTGGAAAGA | D A S AATGCATAAA | D F D CTTTGCCAT |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1460 | D E R N TCTCACCGGA A | G W P TTCAGTCGTC | V E Q ACTCATGGTG | V W K E ATTCTCACT | M H K TGATAACCTT | L L P ATTTTGACG |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1400 | F S P D AGGGGAATT | S V V AATAGGTTGT | T H G ATTGATGTTG | D F S L GACGAGTC <u>GG</u> | D N L AATCGCAGAC | I F D T |
| kan(mu) | | | | | T | | CGATACCGAG |
| ORF | | | | | | | |
| kan(wt) | 1340 | E G K L ATCTTGCAT | I G C CCTATGGAAC | I D V TGCCT <u>CGGT</u> G | G R V G AGTTTCTCC | I A D TTCATTACAG | R Y Q AAACGGCTTT |
| kan(mu) | | | | T | | T | |
| ORF | | | | | | | |
| kan(wt) | 1280 | D L A I TTCAAAAATA | L W N TGGTATTGAT | C L G AATCTGATA | E F S P TGAATAAATT | S L Q GCAGTTTCAT | K R L TTGATGCTCG |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1220 | F Q K Y ATGAGTTTT | G I D CTAATCAGAA | N P D TTGGTTAATT | M N K L GGTTGTAACA | Q F H CTGGCAGAGC | L M L ATTACGCTGA |
| kan(mu) | | | | | | | |
| ORF | | | | | | | |
| kan(wt) | 1160 | D E F F CTTGACGGGA AC | <u>CGCGCA</u> AGC AA AC | TCATGACCAA | AATCCCTAA | CGTGAGTTT | CGTCCACTG |
| kan(mu) | | | | | | | |
| kan(wt) | 1100 | AGCGTCAGAC | CCCGTAGAAA | AGATCAAAGG | ATCTCTTGA | GATCCTTTT | TTCTGGCGGT |
| kan(wt) | 1040 | AATCTGCTGC | TTGCAAACAA | AAAACCACC | GCTACCAAGCG | GTGTTTGTT | TGCCGATCA |
| kan(wt) | 980 | AGAGCTACCA | ACTTTTTC | CGAAGGTAAAC | TGGCTTCAGC | AGAGCGCAGA | TACCAAATAC |
| kan(wt) | 920 | TGTTCTTCTA | GTGTAGCCGT | AGTTAGGCCA | CCACTTCAAG | AACTCTGTAG | CACCGCCTAC |
| kan(wt) | 860 | ATACCTCGCT | CTGCTAATCC | TGTTACCAGT | GGCTGCTGCC | | |

Note: Mutated nucleotides are underlined. The *AlwNI* and *EcoO109I* sites are indicated in bold type. The nucleotide numbering scheme is the same as the backbone vector pUK21.

Please re-write Table 3, beginning on page 60, line 1, as follows:

Plasmid DNA Vectors

Davis *et al.* (1998)

Table 3
Plasmids containing immunostimulatory CpG motifs

| Plasmid | Backbone | No. CpG Motifs | Species Specificity and ODN Equivalence of CpG-S Insert |
|----------|----------|----------------|---|
| pMCG-16 | pMAS | 16 | mouse-specific CpG motif #1826 ¹ |
| pMCG-50 | pMAS | 50 | |
| pMCG-100 | pMAS | 100 | |
| pMCG-200 | pMAS | 200 | |
| pHCG-30 | pMAS | 30 | human-specific CpG motif - no ODN equivalent ² |
| pHCG-50 | pMAS | 50 | |
| pHCG-100 | pMAS | 100 | |
| pHCG-200 | pMAS | 200 | |
| pHIS-40 | pMAS | 40 | human-specific CpG motif #2006 ³ |
| pHIS-64 | pMAS | 64 | |
| pHIS-128 | pMAS | 128 | |
| pHIS-192 | pMAS | 192 | |

¹ sequence of 1826 is TCCATGACGTTCCTGACGTT (SEQ ID NO:51)

² sequence used as a source of CpG motifs is
GACTTCGTGTCGTTCTCTGTCGTCTTAGGCGCTTCTCCTGCGTGCGTCCCTTG (SEQ ID NO:14)

³ sequence of 2006 is TCGTCGTTTGTCGTTTGTCGTT (SEQ ID NO:3)

Please re-write Table 4, beginning on page 61, line 1, as follows:

Table 4

Plasmids encoding hepatitis B surface antigen (derived from ayw or adw subtypes of HBV)

| Plasmid | Backbone | Insert |
|---------------|-----------|--------------|
| pUK-S | pUK21-A2 | HBV-S (ayw) |
| pUKAX-S | pUK21-AX* | HBV-S (ayw) |
| pMAS-S | pMAS | HBV-S (ayw) |
| pMCG16-S | pMCG-16 | HBV-S (ayw) |
| pMCG50-S | pMCG-50 | HBV-S (ayw) |
| pMCG100-S | pMCG-100 | HBV-S (ayw) |
| pMCG200-S | pMCG-200 | HBV-S (ayw) |
| pHCG30-S | pHCG-30 | HBV-S (ayw) |
| pHCG50-S | pHCG-50 | HBV-S (ayw) |
| pHCG100-S | pHCG-100 | HBV-S (ayw) |
| pHCG200-S | pHCG-200 | HBV-S (ayw) |
| pHIS40-S(ad) | pHIS-40 | HBV-S (adw2) |
| pHIS64-S(ad) | pHIS-64 | HBV-S (adw2) |
| pHIS128-S(ad) | pHIS-128 | HBV-S (adw2) |
| pHIS192-S(ad) | pHIS-192 | HBV-S (adw2) |

*pUK21-AX was created by deleting f1 origin from pUK21-A

Please re-write Table 5, beginning on page 62, line 1, as follows:

Table 5 Sequence comparison of pUK21-A2 (SEQ ID NO:83) and pGT (SEQ ID NO:84). 75 point-mutations (indicated with *) in pUK21-A2 results in the gene therapy vector (pGT)

| | |
|------------------------|---|
| pUK21-A2 (1) pGT | GAATTCGAGC TCCCGGGTAC CATGGCATGC ATCGATAGAT CTCGAGTCTA GACTAGAGCT GAATTCGAGC TCCCGGGTAC CATGGCATGC ATCGATAGAT CTCGAGTCTA GACTAGAGCT |
| pUK21-A2 (61) pGT | CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC |
| pUK21-A2 (121) pGT | GTGCCCTCCT TGACCCCTGGA AGGTGCCACT CCCACTGTCC TTTCTTAATA AAATGAGGAA GTGCCCTCCT TGACCCCTGGA AGGTGCCACT CCCACTGTCC TTTCTTAATA AAATGAGGAA |
| pUK21-A2 (181) pGT | ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC |
| pUK21-A2 (241) pGT | AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGAAAGGCCT CGGACTAGTG AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGAAAGGCCT CGGACTAGTG |
| pUK21-A2 (301) pGT | GCGTAATCAT GGTCATAGCT GTTCCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC CCGGAATCAT GGTCATAGCT GTTCCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC |
| pUK21-A2 (361) pGT | *----- AACATACGAG CCGCGGAAGC ATAAAGTGTAAAGCCTGGGG TGCTTAATGA GTGAGCTAAC AACATCCGGG CCGCGGAAGC ATAAAGTGTAAAGCCTGGGG TGCTTAATGA GTGAGCTAAC |
| pUK21-A2 (421) pGT | *----- TCACATTAAT TGCGTTGCGC TCAC TGCCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TCACATTAAT TCGTTGCGC TCAC TGCCCCG CTTTCCAGTC GGGAAACCTG CCGTGCCAGC |
| pUK21-A2 (481) pGT | *----- TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGGTT GCGTATTGGG CGCTCTCCG TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGGTT CGGTATTGGC CGCTCTCCG |
| pUK21-A2 (541) pGT | *----- CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC |
| pUK21-A2 (601) pGT | *----- ACTCAAAGGC GGTAAATACGG TTATCCACAG ATCAGGGGA TAACGCAGGA AAGAACATGT ACTCAAAGGC GGTAAATACGG TTATCCACAG ATCAGGGGA TAACGCAGGA AAGAACATGT |
| pUK21-A2 (661) pGT | *----- GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTCC GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTCC |
| pUK21-A2 (721) pGT | *----- ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA |
| pUK21-A2 (781) pGT | *----- ACCCGACAGG ACTATAAAGA TACCAAGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC ACCCGACAGG ACTATAAAGA TACCAAGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC |
| pUK21-A2 (841) pGT | *----- CTGTTCCGAC CCTGCCGCTT ACGGGATACC TGTCCGCTT TCTCCCTTCG GGAAGCGTGG CTGTTCCGAC CCTGCCGCTT ACGGGATACC TGTCCGCTT TCTCCCTTCG GGAAGCGTGG |
| pUK21-A2 (901) pGT | *----- CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC |
| pUK21-A2 (961) pGT | *----- TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC |
| pUK21-A2 (1021) pGT | *----- GTCTTGAGTC CAACCCGGTA AGACACGACT TATGCCACT GGCAGCAGCC ACTGGTAACA TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC |
| pUK21-A2 (1081) pGT | *----- GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT |
| pUK21-A2 (1141) pGT | *----- ACGGCTACAC TAGAAGAACAA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG ACGGCTACAC TAGAAGAACAA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG |
| pUK21-A2 (1201) pGT | *----- AAAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT AAAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT |
| pUK21-A2 (1261) pGT | *----- AAAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT AAAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT |
| pUK21-A2 (1321) pGT | *----- TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA |

| | | | | | | |
|-----------------|-------------|--------------|-------------|-------------|-------------|-------------|
| pUK21-A2 (1381) | GCTTGCAGCG | TCCCCTCAAG | TCAGCGTAAT | GCTCTGCCAG | TGTTACAACC | AATTAACCAA |
| pGT | GCTTGCAGCG | TCCCCTCAAG | TCACCGGAAT | GCTCTGCCAG | TGTTACAACC | AATTAACCAA |
| pUK21-A2 (1441) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | TTCTGATTAG | AAAAACTCAT | CGAGCATCAA | ATGAAACTGC | AATTTATTCA | TATCAGGATT |
| -----*----- | TTCTGATTAG | AAAAACTCAT | CCAGCATCAA | ATGAAACTGC | AATTTATTCA | TATCAGGATT |
| pUK21-A2 (1501) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | ATCAATACCA | TATTTTGAA | AAAGCCGTTT | CTGTAATGAA | GGAGAAAACT | CACCGAGGCA |
| -----*----- | ATCAATACCA | TATTTTGAA | AAAGCCGTTT | CTGTAATGAA | GGAGAAAACT | CACCGAGGCA |
| pUK21-A2 (1561) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | GTTCCATAGG | ATGGCAAGAT | CCTGGTATCG | GTCTGCATT | CCGACTCGTC | CAACATCAAT |
| -----*----- | GTTCCATAGG | ATGGCAAGAT | CCTGGTATCG | GTCTGCATT | CCGACTCGGC | CAACATCAAT |
| pUK21-A2 (1621) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | ACAACCTATT | AATTCCTCC | CGTAAAAAT | AAGGTTATCA | AGTGAGAAAT | CACCATGAGT |
| -----*----- | ACAACCTATT | AATTCCTCC | CATCAAAAT | AAGGTTATCA | AGTGAGAAAT | CACCATGAGT |
| pUK21-A2 (1681) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | GACGACTGAA | TCCGGTGAGA | ATGGCAAAAG | TTTATGCATT | TCTTCCAGA | CTTGTCAAC |
| -----*----- | AACTACTGAA | TCCGGTGAGA | ATGGCAAAAG | TTTATGCATT | TCTTCCAGA | CTTGTCAAC |
| pUK21-A2 (1741) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | AGGCCAGCCA | TTACGCTCGT | CATCAAAATC | ACTCGCATCA | ACCAAACCGT | TATTCAATTG |
| -----*----- | AGGCCAGCCA | TTACGCTCAT | CATCAAAATC | GGAAGCATCA | ACCAAACCGT | TATTCAATTG |
| pUK21-A2 (1801) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | TGATTGCGCC | TGAGCGAGAC | GAAATACGCG | ATCGCTGTTA | AAAGGACAAT | TACAAACAGG |
| -----*----- | GGATTGAGCC | TGAGCCAGAC | GGAAATACGCG | GTCGCTGTTA | AAAGGACAAT | TACAAACAGG |
| pUK21-A2 (1861) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | AATCGAATGC | AACCGGGCGCA | GGAAACACTGC | CAGCGCATCA | ACAAATTTT | GAGGTGAATC |
| -----*----- | AATGGAATGC | AACCGGGCGGA | GGAAACACTGC | CAGAGCATCA | ACAATTTT | CACCTGAATC |
| pUK21-A2 (1921) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | AGGATATTCT | TCTAATACCT | GGAAATGCTGT | TTTTCCGGGG | ATCGCAGTGG | TGAGTAACCA |
| -----*----- | AGGATATTCT | TCTAATACCT | GGAAATGCTGT | TTTTCCGGGG | ATAGCAGTGG | TGAGTAACCA |
| pUK21-A2 (1981) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | TGCATCATCA | GGAGTACGGA | AAAAATGCTT | GATGGTCGGA | AGAGGCATAA | ATTCCGTCAG |
| -----*----- | TGCATCATCA | GGAGTACGGA | AAAAATGCTT | GATGGTCGGA | AGAGGCATAA | ATTCCGTCAG |
| pUK21-A2 (2041) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | CCAGTTTAGT | CTGACCACATCT | CATCTGTAAC | ATCATGGCA | ACGCTACCTT | TGCCATGTT |
| -----*----- | CCAGTTTAGT | CTGACCACATCT | CATCTGTAAC | ATCATGGCA | ACGCTACCTT | TGCCATGTT |
| pUK21-A2 (2101) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | CAGAAACAAAC | TCTGGCGCAT | CGGGCTTCCC | ATACAAGCGA | TAGATTGTCG | CACCTGATTG |
| -----*----- | CAGAAACAAAC | TCCGGCGCGT | CGGGCTTCCC | ATACAAGCGG | TAGATTGTCG | CACCTGATTG |
| pUK21-A2 (2161) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | CCCGACATTA | TCGCGAGCCC | ATTTATACCC | ATATAAATCA | GCATCCATGT | TGGAATTAA |
| -----*----- | CCCGACATTA | TCGCGAGCCC | ATTTATACCC | ATATAAATCA | GCATCCATGT | TGGAATTAA |
| pUK21-A2 (2221) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | TCGCGGCCTC | GACGTTTCCC | GTTGAATATG | GCTCATAAAC | CCCCTTGTAT | TACTGTTAT |
| -----*----- | TCGCGGCCTG | GAGGTTTCCC | GTTGAATATG | GCTCATAAAC | CCCCTTGTAT | TACTGTTAT |
| pUK21-A2 (2281) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | GTAAGCAGAC | AGTTTTATTG | TTCATGATGA | TATATTTTTA | TCTTGTGCAA | TGTAACATCA |
| -----*----- | GTAAGCAGAC | AGTTTTATTG | TTCATGATGA | TATATTTTTA | TCTTGTGCAA | TGTAACATCA |
| pUK21-A2 (2341) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | GAGATTTGAA | GACACAACGT | GGCTTCCCC | CCCCCCCCCA | TGACATTAAC | CTATAAAAT |
| -----*----- | GAGATTTGAA | GACACACCGG | GGCTTCCCC | CCCCCCCCCA | TGACATTAAC | CTATAAAAT |
| pUK21-A2 (2401) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | AGGCATATCA | CGAGGCCCTT | TCGCTCGCG | CGTTTCGGTG | ATGACGGTGA | AAACCTCTGA |
| -----*----- | AGCCGTATCC | CGAGGCCCTT | CCGCTCGCG | CGTTCCGGTG | ATGCCGGTGA | AAACCTCTGA |
| pUK21-A2 (2461) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | CACATGCAGC | TCCCCTGAGAC | GGTCACAGCT | TGTCGTAAG | CGGATGCCGG | GAGCAGACAA |
| -----*----- | CACATGCAGC | TCCCCTGAGAC | GGTCACAGCT | TGTCGTAAG | CGGATGCCGG | GAGCAGACAA |
| pUK21-A2 (2521) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | GCCCCTGAGG | GCGCGTCAGC | GGGTGTTGGC | GGGTGTCGGG | GCTGGCTTAA | CTATGCGGCA |
| -----*----- | GCCCCTGAGG | GCGCGTCAGC | GGGTGTTGGC | GGGTGTCGGG | GCTGGCTTAA | CTATGCGGCA |
| pUK21-A2 (2581) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | TCAGAGCAGA | TTGTAATGAG | AGTGCACCAT | AAAATTGTAA | ACGTTAATAT | TTTGTAAAAA |
| -----*----- | TCAGAGCAGA | TTGTAATGAG | AGTGCACCAT | AAAATTGTAA | CCGTTAATAT | TTTGTAAAAA |
| pUK21-A2 (2641) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | TTCGCGTTAA | ATTTTGTTA | AATCAGCTCA | TTTTTAACCA | AATAGACCGA | AATCGGCAAA |
| -----*----- | TTCGCGTTAA | ATTTTGTTA | AATCAGCTCA | TTTTTAACCA | AATAGACCGA | AATCGGCAAA |
| pUK21-A2 (2701) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | ATCCCTTATA | AATCAAAAGA | ATAGCCCGAG | ATAGAGTTGA | GTTGTTGTTCC | AGTTTGGAAC |
| -----*----- | ATCCCTTATA | AATCAAAAGA | ATAGCCCGAG | ATAGAGTTGA | GTTGTTGTTCC | AGTTTGGAAC |
| pUK21-A2 (2761) | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- | -----*----- |
| pGT | AAGAGTCCAC | TATTAAGAGA | CGTGGACTCC | AACGTCAAAG | GGCGAAAAAC | CGTCTATCAG |
| -----*----- | AAGAGTCCAC | TATTAAGAGA | CGTGGACTCC | AACGTCAAAG | GGCGAAAAAC | CGTCTATCAG |

| | |
|------------------------|---|
| pUK21-A2 (2821) pGT | GGCGATGGCC CACCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGAACGT GGCAGAGAAAG GCCGATGGCC CACCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGCGCGT GGCAGAGAAAG |
| pUK21-A2 (2881) pGT | -----*----- GAAGGGAAGA AAGCGAAAGG AGCGGGCGCT AAGGCCTGAG CAAGTGTAGC GGTCAACGCTG GAAGGGAAGA AACCGAAAGG AGCGGGCGCT AAGGCCTGAG CAAGTGTAGC GGTCCCGCTG |
| pUK21-A2 (2941) pGT | -----*-----*-----*-----*----- CGCGTAACCA CCACACCCGC CGCGCTTAAT CGCCGCTAC AGGGCGCGTA CTATGGTTGC CGCGTAACCA CCACACCCGC CGCGCTTAAT CGCCGCTAC AGGGCGCGTA CTATGGTTGC |
| pUK21-A2 (3001) pGT | -----*----- TTTGACGTAT GCGGTGTGAA ATACCGCACA GATCGCTAAG GAGAAAATAC CGCATCAGGC TTTGCGTAT GCGGTGTGAA ATACCGCACA GATCGCTAAG GAGAAAATAC CGCATCAGGC |
| pUK21-A2 (3061) pGT | -----*----- GCCATTCGCC ATTCAAGGCT CGCAACTGTT GGGAAAGGCG ATCGGTGCAG GCCTCTTCGC GCCATCCGCC ATTCAAGGCT CGCAACTGTT GGGAAAGGCG ATCGGTGCAG GCCTCTCCGC |
| pUK21-A2 (3121) pGT | -----*----- TATTACGCCA GCTGGCGAA GGGGGATGTG CTGCAAGGCG ATTAAGTTGG GTAACGCCAG TATTACGCCA GCTGGCGAA GGGGGATGTG CTGCAAGGCG ATTAAGTTGG GTAACGCCAG |
| pUK21-A2 (3181) pGT | -----*-----*-----*-----*----- GGTTTCCCA GTCACGACGT TGTAACCGA CGGCCAGTGA ATTGTAATAC GACTCACTAT GGTTTCCCA GTCACGGCGG TGTAACCGA CGGCCAGTGA ATTGTAATCC GACTCACTAT |
| pUK21-A2 (3241) pGT | -----*-----*-----*-----*----- AGGGCGAATT GGGGATCGAT CCACTAGTTC TAGATCCGAT GTACGGGCCA GATATACGCG AGGGCGAATT GGGGACCGAT CCACTAGTTC TAGATCCGAT GTACGGGCCA GATATACGCG |
| pUK21-A2 (3301) pGT | -----*-----*-----*-----*----- TTGACATTGA TTATTGACTA GTTATTAAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG TTGACATTGA TTATTGACTA GTTATTAAATA GTAATCAATT ACGGGGTCAT TAGTTGATAG |
| pUK21-A2 (3361) pGT | -----*-----*-----*-----*----- TTGACATTGA TTATTGACTA GTTATTAAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG TTGACATTGA TTATTGACTA GTTATTAAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG |
| pUK21-A2 (3421) pGT | -----*-----*-----*-----*----- CAACGACCCC CGCCCATGTA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG CAACGACCCC CGCCCATGTA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG |
| pUK21-A2 (3481) pGT | -----*-----*-----*-----*----- GACTTTCCAT TGACGTCAAT GGGTGGAGTA TTTACGGTAA ACTGCCACT TGGCAGTACA GACTTTCCAT TGACGTCAAT GGGTGGAGTA TTTACGGTAA ACTGCCACT TGGCAGTACA |
| pUK21-A2 (3541) pGT | -----*-----*-----*-----*----- TCAAGTGTAT CATATGCCAA GTACGCCCCC TATTGACGTC AATGACGGTA AATGGCCCGC TCAAGTGTAT CATATGCCAA GTACGCCCCC TATTGACGTC AATGACGGTA AATGGCCCGC |
| pUK21-A2 (3601) pGT | -----*-----*-----*-----*----- CTGGCATTAT GCCCAGTACA TGACCTTATG GGACTTTCCCT ACTTGGCAGT ACATCTACGT CTGGCATTAT GCCCAGTACA TGACCTTATG GGACTTTCCCT ACTTGGCAGT ACATCTACGT |
| pUK21-A2 (3661) pGT | -----*-----*-----*-----*----- ATTAGTCATC GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCAGTGGATA ATTAGTCATC GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCAGTGGATA |
| pUK21-A2 (3721) pGT | -----*-----*-----*-----*----- GCGGTTTGAC TCACGGGGAT TTCAAGTCT CCACCCCAT GACGTCATG GGAGTTTGTT GCGGTTTGAC TCACGGGGAT TTCAAGTCT CCACCCCAT GACGTCATG GGAGTTTGTT |
| pUK21-A2 (3781) pGT | -----*-----*-----*-----*----- TTGGCACCAA AATCAACGGG ACTTCCAAA ATGTCGTAAC AACTCCGCC CATTGACGCA TTGGCACCAA AATCAACGGG ACTTCCAAA ATGTCGTAAC AACTCCGCC CATTGACGCA |
| pUK21-A2 (3841) pGT | -----*-----*-----*-----*----- AATGGGCAGGT AGGCCTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG AATGGGCAGGT AGGCCTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG |
| pUK21-A2 (3901) pGT | -----*-----*-----*-----*----- AGAACCCACT GCTTACTGGC TTATCGAAAT TGCGGCCGCC ACGGCGATAT CGGATCCATA AGAACCCACT GCTTACTGGC TTATCGAAAT TGCGGCCGCC ACGGCGATAT CGGATCCATA |
| pUK21-A2 (3961) pGT | -----*-----*-----*-----*----- TGACGTCGAC GCGCTCGCAG AAGCTTC TGACGTCGAC GCGCTCGCAG AAGCTTC |

0965104-096504

Please re-write Table 6, beginning on page 64, line 1, as follows:

Table 6 ODN used with plasmid DNA

| Backbone | ODN code number | Sequence |
|----------------|-----------------|--|
| S-ODN | 1826 | TCCATGAC <u>G</u> TTCCCTGAC <u>G</u> T (SEQ ID NO:51) |
| | 1628 | GGGGTCAAC <u>G</u> TTGAGGGGGG (SEQ ID NO:52) |
| | 1911 | TCCAGGACTTCCCTCAGGTT (SEQ ID NO:53) |
| | 1982 | TCCAGGACTTCTCTCAGGTT (SEQ ID NO:54) |
| | 2017 | CCCCCCCCCCCCCCCCCCCC (SEQ ID NO:55) |
| O-ODN | 2061 | TCCATGAC <u>G</u> TTCCCTGAC <u>G</u> T (SEQ ID NO:56) |
| | 2001 | GG <u>G</u> GGCGGCGGCGGCGG (SEQ ID NO:57) |
| SOS-ODN | 1980 | TCCATGAC <u>G</u> TTCCCTGAC <u>G</u> T (SEQ ID NO:58) |
| | 1585 | GGGGTCAAC <u>G</u> TTGAGGGGGG (SEQ ID NO:59) |
| | 1844 | T <u>C</u> TECC <u>A</u> G <u>C</u> GT <u>G</u> CCATAT (SEQ ID NO:60) |
| | 1972 | GGGGTCTGTGCTTTGGGGGG (SEQ ID NO:61) |
| | 2042 | TCAGGGGTGGGGGGAACCTT (SEQ ID NO:62) |
| | 1981 | GGGGTTGAC <u>G</u> TTTGGGGGG (SEQ ID NO:63) |
| | 2018 | TCTAG <u>C</u> GTTTTAG <u>C</u> GTTCC (SEQ ID NO:64) |
| | 2021 | <u>T</u> CG <u>T</u> CGTTGT <u>C</u> GTT <u>T</u> GT <u>C</u> GTT (SEQ ID NO:65) |
| | 2022 | <u>T</u> CG <u>T</u> CGTTGT <u>C</u> GTT <u>T</u> GT <u>C</u> GTT (SEQ ID NO:66) |
| | 2023 | <u>T</u> CG <u>T</u> CGTTGT <u>C</u> GTT <u>T</u> GT <u>C</u> GTT (SEQ ID NO:67) |

SOS-ODN had two S-linkages at the 5' end, five S-linkages at the 3' end, and O-linkages in between.

Three ODN used in this study were of the same murine-specific immunostimulatory sequence in three different backbones (1826, 2061 and 1980).

All ODN were synthesized by Hybridon (Milford, MA) or Operon (Alameda, CA). ODN were ethanol precipitated and resuspended in saline prior to use alone or as an additive to the plasmid DNA solution.

0051055200

Please re-write Table 10 beginning on page 68, line 1, as follows:

Table 10

Inhibitory CpG motifs can block B cell proliferation induced by a stimulatory CpG motif

| Oligonucleotide added | cpm |
|---|--------|
| medium | 194 |
| 1668 (TCCATGACGTTCCCTGATGCT) (SEQ ID NO:68) | 34,669 |
| 1668 + 1735 (GCGTTTTTTTGCG) (SEQ ID NO:69) | 24,452 |
| 1720 (TCCATGAGCTTCCTGATGCT) (SEQ ID NO:70) | 601 |
| 1720 + 1735 | 1109 |

Splenic B cells from a DBA/2 mouse were cultured at 5×10^4 cells/100 μ l well in 96 well microtiter plates in RPMI as previously described (Krieg, *et al.*, 1995) with or without the indicated phosphorothioate modified oligonucleotides at a concentration of 60 ng/ml for 48 hr. The cells were then pulsed with 3 H thymidine, harvested, and the cpm determined by scintillation counting. The stimulatory CpG oligo 1668 was slightly but significantly inhibited by the inhibitory motifs in oligo 1735. The non CpG oligo 1720 is included as a negative control.

B-96-010-109-00

Please re-write Table 11, beginning on page 69, line 1, as follows:

Table 11

Inhibitory effects of "bad" CpG motifs on the "good" CpG Oligo 1619

Notes:

The sequence of oligo 1619 is TCCATGTCCGTTCCTGATGCT (SEQ ID NO:71)

1949 has only 1 GCG at the 3' end, which has essentially no inhibitory activity

| Oligonucleotide added | IL-12 in pg/ml |
|--|----------------|
| medium | 0 |
| 1619 alone | 6 |
| 1619 + 1949 (TCCATGTC <u>CG</u> TTCCTGATGCG) (SEQ ID NO:72) | 16 |
| 1619 + 1952 (TCCATGTC <u>CG</u> TTC <u>CG</u> CGCGCG) (SEQ ID NO:73) | 0 |
| 1619 + 1953 (TCCATGTC <u>CG</u> TTCCT <u>CG</u> CGCG) (SEQ ID NO:74) | 0 |
| 1619 + 1955 (GCGGCGGGCGGCGCGCGCCC) (SEQ ID NO:75) | 0 |

Human PBMC were cultured in 96 well microtiter plates at 10^5 /200 μ l for 24 hr in RPMI containing 10% autologous serum. Supernatants were collected at the end of the culture and tested for IL-12 by ELISA. All wells except the control (medium) contained 60 μ g/ml of the stimulatory CpG oligodeoxynucleotide 1619; stimulatory (1949) and inhibitory (all other sequences have a strong inhibitory motif) oligos were added to the indicated wells at the same concentration at the beginning of culture. All oligos have unmodified backbones.

D
S
E
L
T
D
G
G
E
H

Please re-write Table 13 beginning on page 71, line 1, as follows:

Table 13 Identification of neutralizing CpG motifs which reduce the induction of cytokine secretion by a CpG-S motif in the same ODN (*cis*-neutralization)

| ODN | sequence 5'-3' ¹ | ODN-induced cytokine expression ² | | | |
|------|--|--|-------|---------------|--|
| | | IL-6 ² | IL-12 | IFN- γ | |
| None | | <5 | 206 | 898 | |
| 1619 | TCCATGT <u>CG</u> TTCC <u>TG</u> TGCT (SEQ ID NO:71) | 1405 | 3130 | 4628 | |
| 1952 | ... <u>GG</u> ... <u>GG</u> ... <u>GG</u> (SEQ ID NO:73) | 559 | 1615 | 2135 | |
| 1953 | ... <u>CC</u> ... <u>CC</u> ... <u>CC</u> ... (SEQ ID NO:74) | 577 | 1854 | 2000 | |

¹Dots in the sequence of ODN 1952 and 1953 indicate identity to ODN 1619; CpG dinucleotides are underlined for clarity. ODN without CpG-N or CpG-S motifs had little or no effect on cytokine production. The data shown are representative of 4 experiments.

²All cytokines are given in pg/ml; measured by ELISA on supernatants from DBA/2 spleen cells cultured in 96 well plates at 2 X 10⁷ cells/ml for 24 hr with the indicated ODN at 30 μ g/ml. Std. dev. of the triplicate wells was <7%. None of the ODN induced significant amounts of IL-5

Please re-write Table 14 beginning on page 72, line 1, as follows:

Table 14 Inhibition of CpG-induced cytokine secretion by ODN containing CpG-N motifs

| ODN | sequence 5'-3' | IL-12 secretion ¹ | CpG-S-induced IL-12 secretion ² |
|------|---|------------------------------|--|
| none | | 268 | 5453 |
| 1895 | <u>GCGCGCGCGCGCGCGCG</u> (SEQ ID NO:76) | 123 | 2719 |
| 1896 | <u>CCGGCC<u>GGCG<u>GGCG<u>GGCG</u>GG</u>GG</u> (SEQ ID NO:77)</u> | 292 | 2740 |
| 1955 | <u>GCG<u>GGGGGGGGGGGGGG</u>GGCC (SEQ ID NO:75)</u> | 270 | 2539 |
| 2037 | <u>TCCAT<u>GGCG<u>GGCG<u>GGCG</u>TT</u>CC<u>GGCG</u>TT</u> (SEQ ID NO:78)</u> | 423 | 2847 |

¹BALB/c spleen cells were cultured in 96 well plates at 2×10^7 cells/ml with the indicated ϕ DN for 24 hr and then the supernatants were assayed for IL-12 by ELISA (pg/ml).

²Cells were set up the same as in ¹ except that IL-12 secretion was induced by the addition of the CpG ODN 1619 (TCCATGTCGTTCCCTGATGCT) (SEQ ID NO: 71) at 30 μ g/ml. The data shown are representative of 5 experiments.